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STRUCTURAL FEATURES OF THE MUCILAGE FROM THE STEM PITH OF KIWIFRUIT (*Actinidia deliciosa*): PART II, STRUCTURE OF THE NEUTRAL OLIGOSACCHARIDES

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ABSTRACT

Graded acid hydrolysis of the mucilage from the stem pith of *Actinidia deliciosa* gave a series of neutral oligosaccharides that were fractionated by gel filtration. The methylated alditol derivatives were isolated by reverse-phase h.p.l.c. and characterised by f.a.b.-m.s., e.i.-m.s., and g.l.c.–m.s. of the methylated alditol acetates. The results suggest the glucuronomannan core of the mucilage is substituted with oligosaccharides composed of (1→3)-linked β-D-galactose residues that are partially substituted through positions 2 and 6 with Ara*f*, Ara*p*, Fuc*p*, and Gal*p*. A tentative structure for the mucilage is proposed.

INTRODUCTION

In the preceding paper[1], methylation analysis demonstrated that the backbone of the mucilage from *Actinidia deliciosa* was composed of the repeating unit →4)-β-D-Glc*p*A-(1→2)-D-Man*p*-(1→. About 50% of the glucosyluronic acid residues and 95% of the mannosyl residues are substituted through positions 3 with oligosaccharides containing β-D-galactose, L-arabinose, and α-L-fucose. We now provide evidence for the structures of the neutral side-chains of the mucilage.

RESULTS

Graded acid hydrolysis. — Partial acid hydrolysis of the mucilage with boiling 50mM oxalic acid gave a degraded polymer (DKM1) together with neutral mono- and oligo-saccharides containing galactose, fucose, and arabinose in the ratios 1.0:3.2:7.9. Only traces of mannose were present.

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Gel filtration of the neutralised hydrolysate on Bio-Gel P-2 (Fig. 1) gave mono- (OA1, 112 mg), di- (OA2, 20.1 mg), tri- (OA3, 4.3 mg), tetra- (OA4, 4.6 mg), penta- (OA5, 3.5 mg), and hexa-saccharide (OA6, 2.3 mg) fractions. Fractions OA2–OA6 were each reduced and methylated, and the products were subjected to reverse-phase h.p.l.c. (Fig. 2, a–d). The purified derivatives were characterised by retention time (*T* relative to that of methylated maltopentaose-alditol) in g.l.c.[2] on OV-1, f.a.b.-m.s.[2], e.i.-m.s.[3–7], and g.l.c.–m.s.[8] of the alditol acetates.

Reverse-phase h.p.l.c. of methylated OA2 revealed a single peak (A, Fig. 2), which in g.l.c. was eluted in the region (*T* 0.2) for a methylated disaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 384, corresponding to $[M + 1]^+$ from a derivative containing pentose and pentitol residues. E.i.-m.s. gave ions at *m/z* 90 (67.8%), 134 (56.7), 175 (aA_1, 72.8), 192 ($aldJ_2$, 73.0), 252 ($aldJ_1$, 54.6), and 384 ($[M + 1]^+$, 64.0). This confirmed the disaccharide nature of the derivative and that the pentitol residue was substituted through position 5.

Linkage analysis of the component in peak A gave the acetylated derivatives of 1,2,3,4-tetra-*O*-methyl- and 2,3,4-tri-*O*-methyl-arabinitol in the ratio 1:1. These data demonstrated that the material in peak A was derived from the disaccharide Ara*p*-(1→5)-Ara*f* (**1**).

Reverse-phase h.p.l.c. of methylated OA3 gave two major components (B and D, Fig. 2b) and one minor component (C).

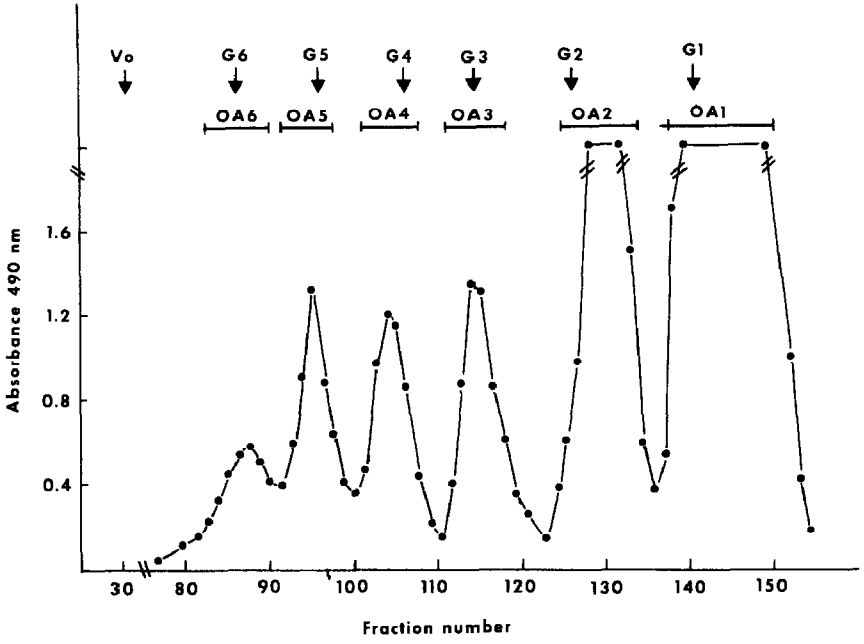

Fig. 1. Fractionation on Bio-Gel P-2 of the neutral material obtained by hydrolysis of the mucilage from *A. deliciosa* with 50mM oxalic acid. The void volume (V_0) was determined with Blue Dextran; G_1, glucose; G_2–G_6, maltose–maltohexose.

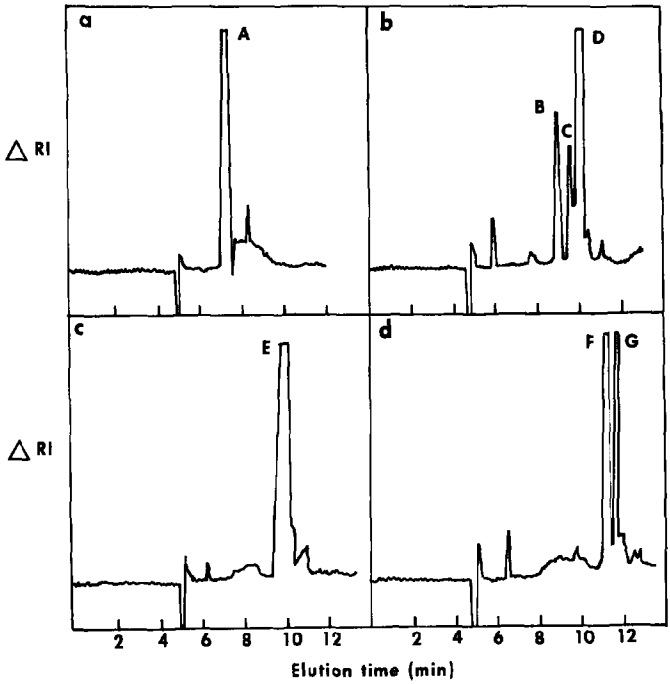

Fig. 2. Reverse-phase h.p.l.c. of the methylated oligosaccharide-alditols derived from fractions OA2–OA5 (Fig. 1); a–d correspond to OA2–OA5.

The component in peak B was eluted in the region (*T* 0.60) for a methylated trisaccharide-alditol in g.l.c. F.a.b.-m.s. gave ions at *m/z* 588 and 676, corresponding to $[M + 1]^+$ from derivatives containing one hexitol and two pentose, and one hexitol and two hexose residues, respectively. E.i.-m.s. gave ions at *m/z* 175 (aA_1, 34.3%), 236 ($aldJ_2$, 3.6), 296 ($aldJ_1$, 0.2), 335 (baA_1, 0.3), and 396 ($baldJ_2$, 0.2) which, in combination with the structure **1**, were consistent with the structure Ara*p*-(1→5)-Ara*f*-(1→3)-Gal*p*. E.i.-m.s. also gave ions at *m/z* 219 (aA_1, 2.9%), 423 (baA_1, 0.3), and 440 ($baldJ_2$, 0.2) suggesting the presence of the sequence Gal*p*-(1→?)-Gal*p*-(1→3)-Gal*p*. That the hexitol was 3-linked in each trisaccharide was indicated by the presence of a relatively intense ion at *m/z* 133 (6.0%) and a weak ion at *m/z* 177 (0.1).

In g.l.c., the component in peak C was eluted in the region (*T* 0.6) for a methylated trisaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 676, corresponding to $[M + 1]^+$ from a derivative containing one hexitol and two hexose residues. E.i.-m.s. gave ions at 133 (10.2%), 219 (aA_1, 18.2), 236 ($aldJ_2$, 4.0), 296 ($aldJ_1$, 0.3), 423 (baA_1, 0.1), 440 ($baldJ_2$, 1.1), and 500 ($baldJ_1$, 0.3) confirming the trisaccharide nature of the derivative. The presence of the $aldJ_1$ ion and the absence of the $aldJ_0$ ion suggested[9] that the internal hexosyl residue was not linked through position 3. Linkage analysis gave the acetylated derivatives of 1,2,4,5,6-penta-*O*-methyl-, 2,3,4,6-tetra-*O*-methyl-, and 2,3,4-tri-*O*-methyl-galactitol in the ratios

1.0:0.9:1.3. These data demonstrated that the component in peak C was derived from the trisaccharide Gal*p*-(1→6)-Gal*p*-(1→3)-Gal*p* (**2**).

In g.l.c., the component in peak D was eluted in the region (*T* 0.60) for a methylated trisaccharide-alditol. F.a.b.-m.s. gave ions at *m/z* 646 and 676, corresponding to $[M + 1]^+$ from derivatives containing hexose, 6-deoxyhexose, and hexitol, and one hexitol and two hexose residues, respectively. E.i.-m.s. gave ions at *m/z* 189 (aA_1, 5.0%) and 393 (baA_1, 0.5), and 219 (aA_1, 9.4) and 423 (baA_1, 0.8), which were consistent with the occurrence of the sequences 6-deoxyhexosyl-(1→?)-hexosyl-(1→?)- and hexosyl-(1→?)-hexosyl-(1→?). Ions at *m/z* 236 ($aldJ_2$, 5.0%), 296 ($aldJ_1$, 0.5), 440 ($baldJ_2$, 0.4), and 500 ($baldJ_1$, 0.2) together with results of linkage analysis, which gave the acetylated derivatives of 1,2,4,5,6-penta-*O*-methyl-, 2,3,4,6-tetra-*O*-methyl-, 2,4,6- and 2,3,4-tri-*O*-methyl-galactitol, and 2,3,4-tri-*O*-methylfucitol, suggested that the mixture contained the two parent oligosaccharides Fuc*p*-(1→6)-Gal*p*-(1→3)-Gal*p* and Gal*p*-(1→3)-Gal*p*-(1→3)-Gal*p*.

Reverse-phase h.p.l.c. (Fig. 2C) of methylated OA4 gave a diffuse peak in g.l.c. which was eluted in the region (*T* 0.80) for a methylated tetrasaccharide-alditol. F.a.b.-m.s. gave ions at *m/z* 850 (minor) and 880 (major), corresponding to $[M + 1]^+$ from derivatives containing one hexitol, one 6-deoxyhexose, and two hexose, and one hexitol and three hexose residues, respectively. E.i.-m.s. gave ions at *m/z* 189 (aA_1, 3.6%), 219 (aA_1, 23.1), and 236 ($aldJ_2$, 11.5) which were consistent with the presence of oligosaccharides containing hexose at the reducing terminus and 6-deoxyhexose and hexose at the non-reducing termini of the parent oligosaccharides. Further evidence for the structure of these oligosaccharides was not obtained.

Reverse-phase h.p.l.c. of methylated OA5 gave two well resolved peaks (F and G, Fig. 2d). In g.l.c., the component in peak F was eluted in the region (*T* 1.04) for a methylated pentasaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 1084, corresponding to $[M + 1]^+$ from a derivative containing a hexitol and four hexose residues. E.i.-m.s. gave ions at *m/z* 133 (21.0%), 219 (aA_1 and $a'A_1$, 67.5), 236 ($aldJ_2$, 48.7), 282 ($aldJ_0$, 1.8), 440 ($caldJ_2$, 1.4), 486 ($caldJ_0$, 0.9), 627 ($baa'A_1$, 0.4), 831 ($cbaa'A_1$, 0.4), and 848 ($bacaldJ_2$, 0.1). These ions, together with the absence of a significant amount of the ion at *m/z* 423 (baA_1), were consistent with the presence of a branched pentasaccharide-alditol derivative. The ratios of intensities of the ions $aldJ_0$ and $aldJ_1$, and $caldJ_0$ and $caldJ_1$, were 1.0:0.6 and 1.0:0.4, respectively, suggesting[9] that both internal hexosyl residues were substituted through position 3. The absence of an ion at *m/z* 423 and the presence of an ion at *m/z* 440 suggested that the hexose residue attached to the hexitol moiety was substituted only through position 3. Linkage analysis of the component in peak F gave the acetylated derivatives of 1,2,4,5,6-penta-*O*-methyl-, 2,3,4,6-tetra-*O*-methyl-, 2,4,6-tri-*O*-methyl-, and 2,4-di-*O*-methyl-galactitol in the ratios 1.0:2.0:1.1:1.2. These data suggested that the derivative in peak F was derived from the branched pentasaccharide **3**.

Gal*p*-(1→3)-Gal*p*-(1→3)-Gal*p*-(1→3)-Gal*p*
6
↑
1
Gal*p*

3

In g.l.c., the component in peak G was eluted in the region (*T* 1.0) for a methylated pentasaccharide. F.a.b.-m.s. gave an ion at *m/z* 1054, corresponding to a derivative containing one hexitol, one 6-deoxyhexose, and three hexose residues. E.i.-m.s. gave ions at *m/z* 189 (aA_1, 13.6%), 219 ($a'A_1$, 8.6), and 236 ($aldJ_2$, 7.1) demonstrating the presence of non-reducing terminal fucosyl and galactosyl groups and galactose at the reducing terminus. Further evidence for the structure of the oligosaccharide was not obtained.

In g.l.c., methylated OA6 gave a single peak that was eluted in the region (*T* 1.18) for a methylated hexasaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 1258, corresponding to $[M + 1]^+$ from a derivative containing one hexitol, one 6-deoxyhexose, and four hexose residues. E.i.-m.s. gave ions at *m/z* 189 (aA_1, 17.5%), 219 (aA_1, 15.4), and 236 ($aldJ_2$, 11.7), which suggested that the parent hexasaccharide was branched. This view was confirmed by linkage analysis which gave similar proportions of the acetylated derivatives of 4,6-di-*O*-methyl-, and 2,4-di-*O*-methyl-galactitol. However, due to the absence of diagnostic ions for the larger fragments, further sequence information could not be obtained.

Partial acid hydrolysis of the oxalic acid-degraded mucilage (DKM1) with 0.5M trifluoracetic acid at 100° gave neutral mono- and oligo-saccharides containing arabinose, fucose, and galactose. Only a trace (~1%) of mannose was detected. Gel filtration (Fig. 3) of the neutral fraction on Bio-Gel P-2 yielded mono- (TFA1, 55.5 mg), di- (TFA2, 24.7 mg), tri- (TFA3, 16.4 mg), tetra- (TFA4, 10.2 mg), and penta-saccharide (TFA5, 4.5 mg) fractions.

The oligosaccharides in each of the fractions TFA 2–5 were reduced, methylated, subjected to reverse-phase h.p.l.c. (Fig. 4), and characterised as described for the oligosaccharide-alditol derivatives obtained from the oxalic acid hydrolysate.

The methylated derivatives in fraction TFA2 were separated into a minor and a major peak, respectively, by h.p.l.c. (Fig. 4a). In g.l.c., the minor component (peak I, Fig. 4a) was eluted in the region (*T* 0.20) for a methylated disaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 472, corresponding to $[M + 1]^+$ from a derivative containing hexose and hexitol residues. E.i.-m.s. gave ions at *m/z* 219 (aA_1, 35.3%), 236 ($aldJ_2$, 38.6), 296 ($aldJ_1$, 28.2), 338 (M − 133, 1.6), and 382 (M − 89, 7.8). Linkage analysis of the derivative gave approximately equal amounts of the acetylated derivatives of 1,2,4,5,6-penta-*O*-methylgalactitol and 2,3,4,6-tetra-*O*-methylgalactitol. These data established that the derivative in peak I was obtained from the disaccharide Gal*p*-(1→3)-Gal*p* (**4**).

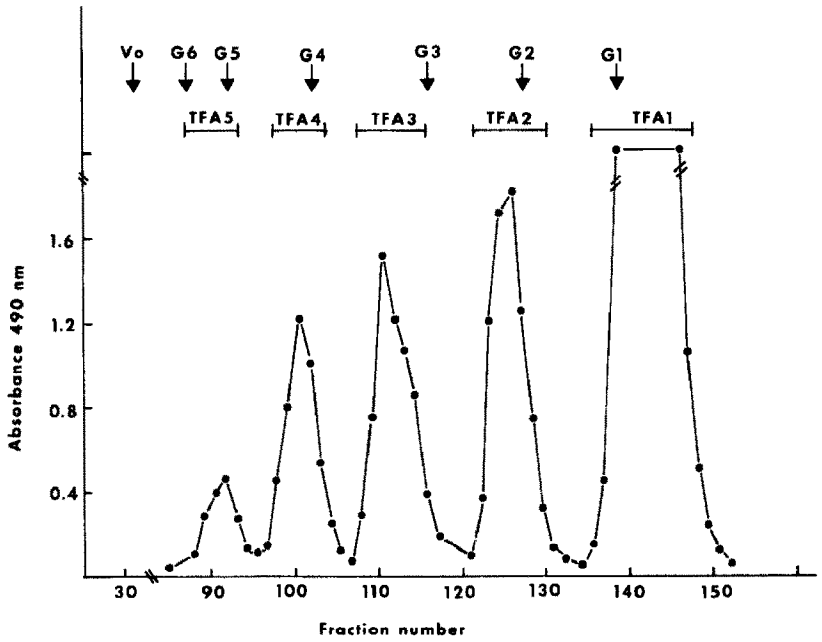

Fig. 3. Fractionation on Bio-Gel P-2 of the neutral material obtained from the hydrolysis with 0.5M trifluoroacetic acid of the oxalic acid-degraded mucilage from *A. deliciosa*. The void volume (V_0) was determined with Blue Dextran; G_1, glucose; G_2–G_6, maltose–maltohexose.

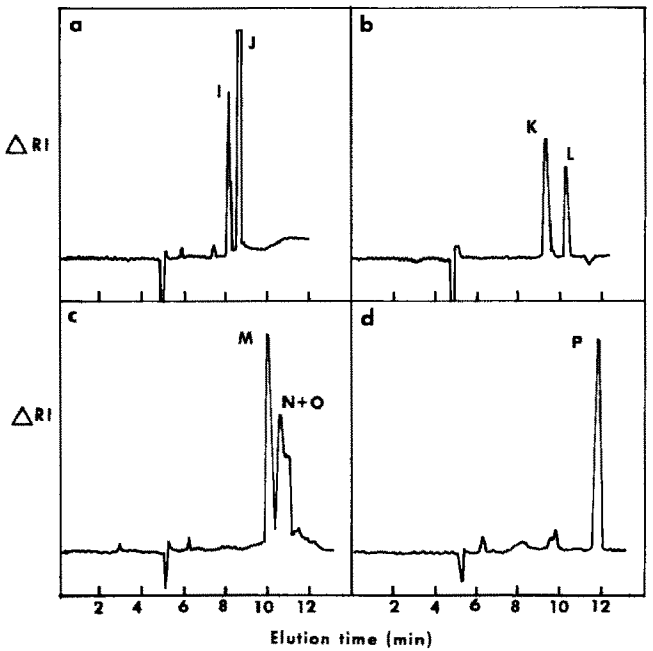

Fig. 4. Reverse-phase h.p.l.c. of the methylated oligosaccharide-alditols derived from fractions TFA2–TFA5 (Fig. 3); a–d correspond to TFA2–TFA5.

In g.l.c., the major component (peak J, Fig. 4a) in fraction TFA2 was eluted in the region (*T* 0.21) for a methylated disaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 472, corresponding to $[M + 1]^+$ from a derivative containing hexose and hexitol residues. E.i.-m.s. gave ions at *m/z* 134 (28.7%), 178 (18.4), 219 (aA_1, 24.1), 236 ($aldJ_2$, 44.1), 293 (M − 178, 0.4), 296 ($aldJ_1$, 0.6), and 337 (M − 134, 3.7). These ions, together with the detection of 1,2,3,4,5-penta-*O*-methylgalactitol and 2,3,4,6-tetra-*O*-methylgalactitol in the ratio of 1:1, after analysis of the methylated alditol acetates by g.l.c.–m.s., showed that the component in peak J was derived from the disaccharide Gal*p*-(1→6)-Gal*p* (**5**).

The methylated oligosaccharide-alditols in fraction TFA3 were resolved by h.p.l.c. into two peaks (K and L, Fig. 4b). In g.l.c., the methylated derivative in peak K was eluted in the region (*T* 0.60) for a methylated trisaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 676, corresponding to $[M + 1]^+$ from a derivative containing one hexitol and two hexose residues. E.i.-m.s. and linkage analysis data were identical to those for trisaccharide **2**, indicating that the component in peak K was also derived from **2**.

In g.l.c., the methylated derivative in peak L was eluted in the region (*T* 0.60) for a methylated trisaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 676, corresponding to $[M + 1]^+$ from a derivative containing one hexitol and two hexose residues. E.i.-m.s. gave ions at *m/z* 219 (aA_1, 53.9%), 236 ($aldJ_2$, 51.3), 282 ($aldJ_0$, 9.7), 423 (baA_1, 33.5), 440 ($baldJ_2$, 9.1), 500 ($baldJ_1$, 3.3), 542 (M − 133, 1.9), and 586 (M − 89, 2.8). The ratio of intensities of the ions $aldJ_0$ to $aldJ_1$ of 1.0:0.7 indicated that the internal hexosyl residue was substituted through position 3. This view was confirmed by linkage analysis, which gave the acetylated derivatives of 1,2,4,5,6-penta-*O*-methyl-, 2,3,4,6-tetra-*O*-methyl-, and 2,4,6-tri-*O*-methylgalactitol in the ratios 1.0:1.2:1.5. These data established that the derivative in peak L was obtained from the trisaccharide Gal*p*-(1→3)-Gal*p*-(1→3)-Gal*p* (**6**).

Reverse-phase h.p.l.c. of the methylated derivatives in fraction TFA4 gave two major peaks (peaks M and N, Fig. 4c). Refractionation of the components in peak N under the same conditions resolved it into two components (peaks N and O).

In g.l.c., the derivative in peak M was eluted in the region (*T* 0.80) for a methylated tetrasaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 880, corresponding to $[M + 1]^+$ from a derivative containing one hexitol and three hexose residues. E.i.-m.s. gave ions at *m/z* 133 (48.3%), 219 (aA_1, 67.8), 286 ($aldJ_2$, 59.1), 282 ($aldJ_0$, 1.7), 627 ($baa'A_1$, 1.5), 644 ($ba'aldJ_2$, 1.2), 704 ($ba'aldJ_1$, 0.1), and 790 (M − 89, 0.4). The ratio of intensities of the ions $aldJ_0$ to $aldJ_1$ of 1.0:0.8 suggested[9] that the residue attached to the hexitol moiety was linked through position 3. Linkage analysis of the derivative gave the acetylated derivatives of 1,2,4,5,6-penta-*O*-methyl-, 2,3,4,6-tetra-*O*-methyl-, and 2,4-di-*O*-methyl-galactitol in the ratios 1.0:2.3:1.1. These data established that the methylated oligosaccharide-alditol in peak M was derived from the branched tetrasaccharide **7**.

Gal*p*-(1→3)-Gal*p*-(1→3)-Gal*p*
6
↑
1
Gal*p*

7

In g.l.c., the component in peak N was eluted in the region (*T* 0.81) for a methylated tetrasaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 880, corresponding to $[M + 1]^+$ from a derivative containing one hexitol and three hexose residues. E.i.-m.s. gave ions at *m/z* 133 (6.4%), 219 (aA_1, 8.1), 236 (*ald*J_2, 4.8), 282 (*ald*J_0, 0.4), 423 (baA_1, 0.4), 440 (*cald*J_2, 0.3), 500 (*cald*J_1, 0.2), and 627 ($cbaA_1$, 0.2). The ratio intensities of the ions *ald*J_0 to *ald*J_1 of 2.0:1.0 suggested[9] that the residue attached to the hexitol was linked through position 3. The presence of the b*ald*J_1 ion and the absence of the b*ald*J_0 ion indicated that the terminal hexosyl group was not linked to position 3 of the penultimate hexosyl residue. This view was confirmed by linkage analysis, which gave the acetylated derivatives of 1,2,4,5,6-penta-*O*-methyl-, 2,3,4,6-tetra-*O*-methyl-, 2,4,6-tri-*O*-methyl-, and 2,3,4-tri-*O*-methyl-galactitol in the ratios 1.0:1.1:1.2:1.5. The component in peak N was therefore derived from the tetrasaccharide **8**.

Gal*p*-(1→6)-Gal*p*-(1→3)-Gal*p*-(1→3)-Gal*p* (**8**).

In g.l.c., the component in peak O was eluted in the region (*T* 0.81) for a methylated tetrasaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 880, corresponding to $[M + 1]^+$ from a derivative containing one hexitol and three hexose residues. E.i.-m.s. gave ions at *m/z* 133 (16.5%), 219 (aA_1, 31.9), 236 (*ald*J_2, 21.9), 282 (*ald*J_0, 1.0), 423 (baA_1, 9.3), 440 (*cald*J_2, 0.9), 486 (*cald*J_0, 0.7), 627 ($cbaA_1$, 1.7), 644 (bc*ald*J_2, 0.1), and 704 (bc*ald*J_1, 0.2). The ratio of intensities of the ions *ald*J_0 to *ald*J_1, and *cald*J_0 to *cald*J_1, of 1.0:0.7 and 1.0:0.1, respectively, suggested[9] that both internal hexosyl residues were 3-linked. Linkage analysis gave the acetylated derivatives of 1,2,4,5,6-penta-*O*-methyl-, 2,3,4,6-tetra-*O*-methyl-, and 2,4,6-tri-*O*-methyl-galactitol in the ratios 1.0:2.0:1.0. These data demonstrated that the component in peak O was derived from the tetrasaccharide **9**.

Gal*p*-(1→3)-Gal*p*-(1→3)-Gal*p*-(1→3)-Gal*p* (**9**).

Reverse-phase h.p.l.c. of the methylated fraction TFA5 gave a single peak (P, Fig. 4d) which, in g.l.c., was eluted in the region (*T* 1.0) for a methylated pentasaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 1084, corresponding to $[M + 1]^+$ from a derivative containing one hexitol and four hexose residues. E.i.-m.s. and linkage analysis data were identical to those for **3**, indicating that the component in peak P was derived from the same pentasaccharide.

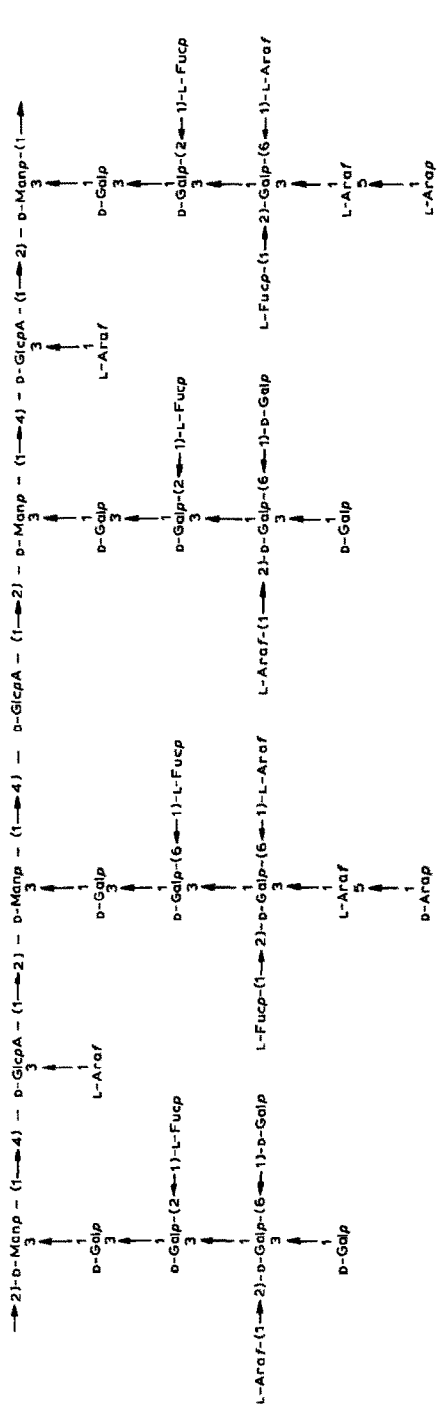

Fig. 5. Tentative structure for a portion of *A. deliciosa* mucilage.

DISCUSSION

In the preceding paper, it was suggested[1] that the glucuronomannan core of the *A. deliciosa* mucilage was substituted through positions 3 of the mannosyl residues with galactose-containing oligosaccharides. The characterisation of an homologous series of oligosaccharides (**4, 6**, and **9**) containing ≤4 (1→3)-linked galactose residues suggested that these were the core of the neutral oligosaccharides. Some of these sequences appear to be branched, as demonstrated by the characterisation of oligosaccharides (**2, 3, 5, 7**, and **8**) containing (1→6)-linked galactose residues. The absence of significant amounts of oligosaccharides containing (1→2)-linked galactose residues indicated that this position was substituted by acid-labile arabinose and fucose residues in the parent polymers.

Hydrolysis of the polysaccharide with 50mM oxalic acid gave a disaccharide **1** containing arabinose. The relatively high yield of this component was attributed to the increased stability to acid hydrolysis of the Ara*p* linkage when compared with the Ara*f* linkage. The isolation of a trisaccharide tentatively identified as Ara*p*-(1→5)-Ara*f*-(1→3)-Gal*p* suggests that **1** was attached to the galactose-containing side-chains and not to position 3 of the glucuronic acid. The substituent on position 3 of the glucuronic acid was probably a single Ara*f* residue.

The combined results of methylation analysis of the intact and partially degraded mucilage[1] and the characterisation of the neutral oligosaccharides released by graded acid hydrolysis allowed a partial structure of the mucilage to be proposed (Fig. 5). The assignment of the anomeric nature of the glycosidic linkages and the absolute configuration of the sugars is based[1] on earlier evidence. The sequence presented in Fig. 5 is only an average structure and does not account for the relative distribution of neutral oligosaccharide side-chains, the occurrence of ~5% of (1→2)-linked mannose, and the occurrence of a small proportion (0.37%) of xylose.

EXPERIMENTAL

The general experimental procedures have been described in the preceding paper[1].

Graded acid hydrolysis of the mucilage. — (*a*) *50mM Oxalic acid.* The mucilage (KM1, 412 mg) in 50mM oxalic acid (200 mL) was heated for 2 h at 100°. The cooled hydrolysate was concentrated to ~50 mL, and the mono- and oligosaccharides were separated from the degraded polymer (DKM1) by dialysis against distilled water. The diffusate was adjusted to pH 7.5 with $Ba(OH)_2$, filtered, and de-salted on columns (1 × 5 cm) of QAE-Sephadex ($HCOO^-$) and SP-Sephadex (H^+) polymers by elution with water. The eluate was concentrated and freeze-dried to give fraction OA (140 mg).

(*b*) The oxalic acid-treated material (DKM1, 245 mg) in 0.5M trifluoroacetic acid (100 mL) was heated for 1 h at 100°. The mono- and oligo-saccharides were

isolated by dialysis, the diffusate was concentrated to dryness, and the residual acid was removed by co-distillation with water (4 × 20 mL). The residue in water was freeze-dried to give fraction TFA (yield, 120 mg).

Gel filtration of the neutral oligosaccharides. — The fractions obtained after graded acid hydrolysis with oxalic acid and trifluoroacetic acid, respectively, in water (2 mL) were eluted from a column (150 × 1 cm) of Bio-Gel P-2 (400 mesh) maintained at 50° with water (40 mL/h). Fractions (0.65 mL) were collected and portions (50 μL) assayed[10] for total carbohydrate by the phenol–sulfuric acid method. Appropriate fractions were combined and freeze-dried.

Methylation of the oligosaccharides. — The oligosaccharides were converted into their corresponding alditols by reduction with NaB^2H_4, de-salted, and methylated[11].

Isolation of the methylated oligosaccharide-alditols. — Sep-Pak C_{18} cartridges, attached to a 5-mL glass syringe, were conditioned[12] by elution with methanol (20 mL), acetonitrile (10 mL), aqueous 60% acetonitrile (10 mL), and water (20 mL).

The reaction mixture containing the methylated oligosaccharide-alditols was diluted with water to give aqueous 20% methyl sulphoxide, and the excess of alkylating reagent was then removed by aspiration with argon. The aqueous mixture (~2.5 mL) was eluted[2] through the conditioned Sep-Pak cartridge with water (10 mL) and aqueous 20% acetonitrile (8 mL). The methylated oligosaccharide-alditols were eluted[2] with aqueous 60% acetonitrile (10 mL), and the eluate was concentrated to dryness at 20°. A solution of the residue in aqueous 60% acetonitrile (200 μL) was filtered through a 0.45-μm fluoropore membrane, using a centrifugal microfilter.

Reverse-phase h.p.l.c. — H.p.l.c. was performed on a Perkin–Elmer Series 2 chromatograph, using[2] a Zorbax ODS column (25 cm × 4.6 mm) with a Brownlee C_{18} guard column. Samples (80 μL) were introduced through a Rheodyne 7125 injector (100-μL loop) and eluted[6] isocratically with aqueous 60% acetonitrile at 0.5 mL/min (~3.5 MPa). Fractions (200 μL) were collected and monitored[2] for methylated oligosaccharide-alditols by g.l.c. on a column (45 cm × 4 mm) containing 1% of OV-1. The appropriate fractions were combined and concentrated to dryness, and a solution of the residue in acetone (50 μL) was used for f.a.b.-m.s.[2] and e.i.-m.s.[2].

Mass spectrometry. — E.i.-m.s. was performed[13] on a Kratos MS80 mass spectrometer. F.a.b.-m.s. was performed[14] on a Kratos M9 mass spectrometer, using xenon as the primary bombarding gas.

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REFERENCES

1 R. J. REDGWELL, M. A. O'NEILL, R. R. SELVENDRAN, AND K. J. PARSLEY, *Carbohydr. Res.*, 153 (1986) 97–106.
2 M. A. O'NEILL AND R. R. SELVENDRAN, *Carbohydr. Res.*, 145 (1985) 45–58.
3 N. K. KOCHETKOV AND O. S. CHIZHOV, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
4 J. KÄRKKÄINEN, *Carbohydr. Res.*, 17 (1971) 1–10.
5 V. KOVACIK, S. BAUER, J. ROSIK, AND P. KOVAC, *Carbohydr. Res.*, 8 (1968) 282–290.
6 M. MCNEIL, A. G. DARVILL, P. AMAN, L.-E. FRANZEN, AND P. ALBERSHEIM, *Methods Enzymol.*, 83 (1981) 3–45.
7 B. NILSSON AND D. ZOPF, *Methods Enzymol.*, 83 (1982) 46–58.
8 M. A. O'NEILL AND R. R. SELVENDRAN, *Carbohydr. Res.*, 79 (1980) 115–124.
9 J. K. SHARP AND P. ALBERSHEIM, *Carbohydr. Res.*, 128 (1984) 193–202.
10 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
11 M. A. O'NEILL AND R. R. SELVENDRAN, *Carbohydr. Res.*, 111 (1983) 239–255.
12 T. J. WAEGE, A. G. DARVILL, M. MCNEIL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 123 (1983) 281–304.
13 D. ASHFORD, N. N. DESAI, A. K. ALLEN, A. NEUBERGER, M. A. O'NEILL, AND R. R. SELVENDRAN, *Biochem. J.*, 201 (1982) 199–208.
14 G. R. FENWICK, J. EAGLES, AND R. SELF, *Org. Mass Spectrom.*, 17 (1982) 544–546.